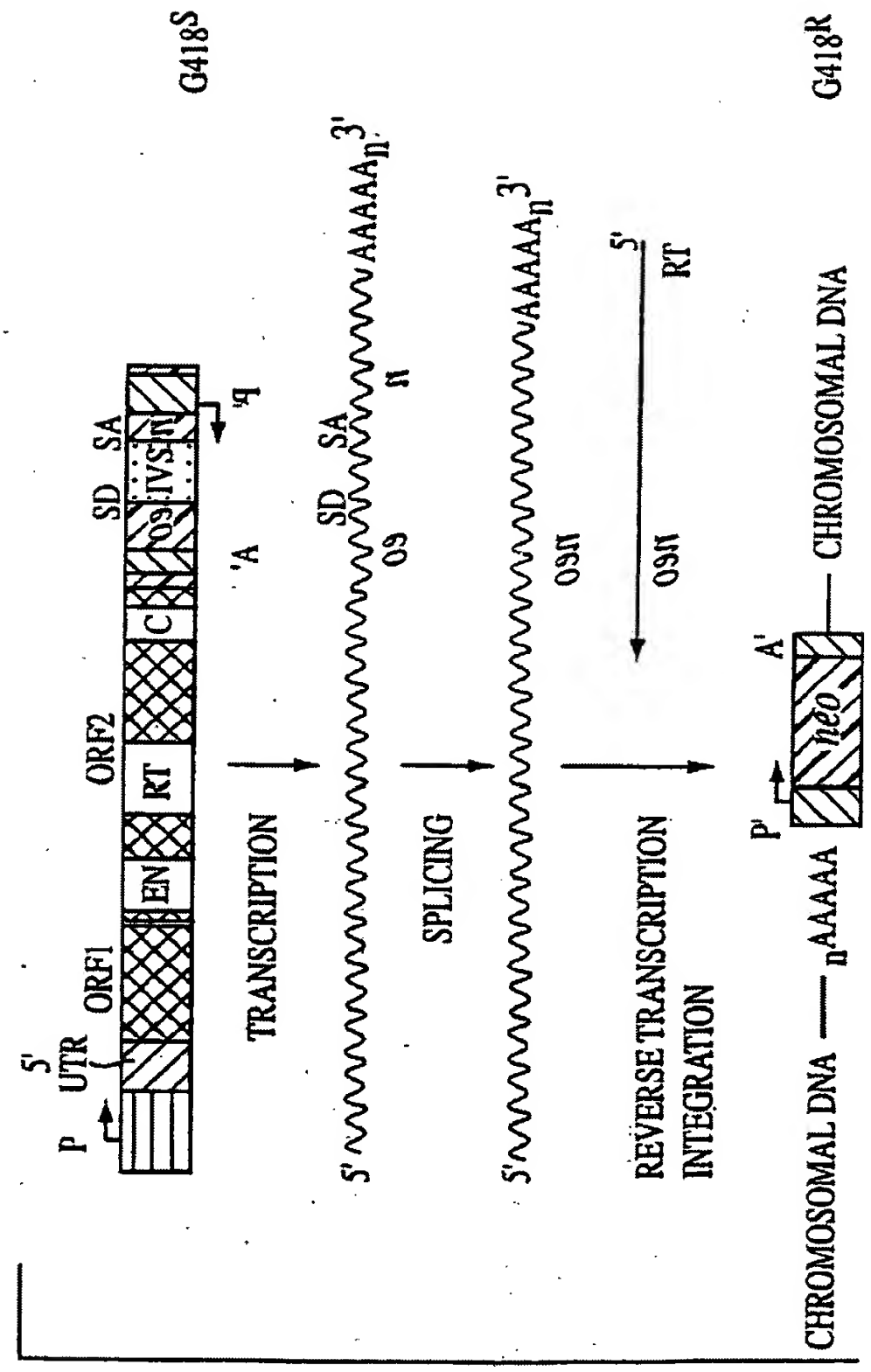
FIG. 1A


FIG. 1B



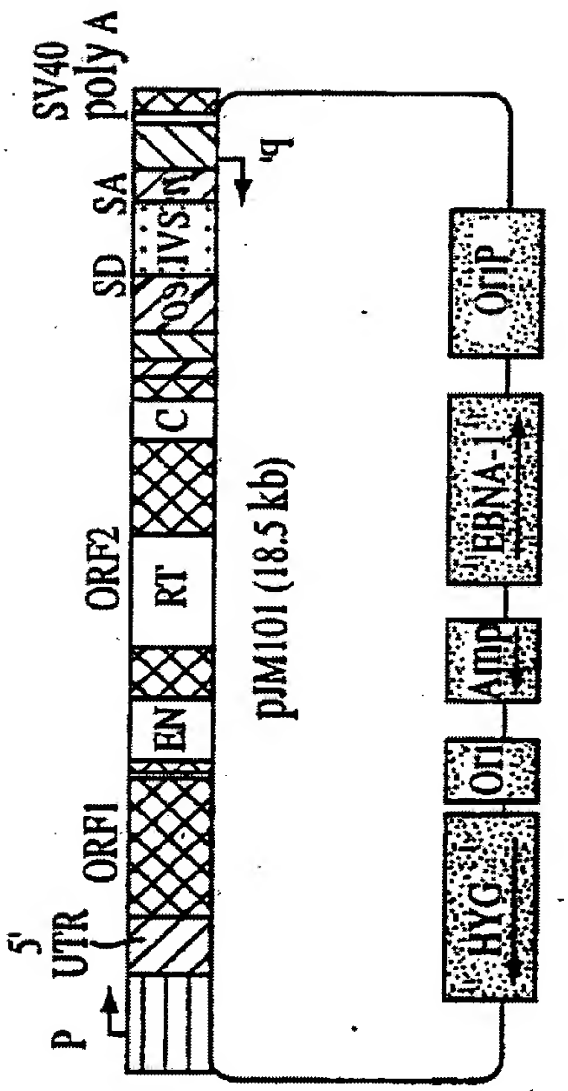



FIG. 2A

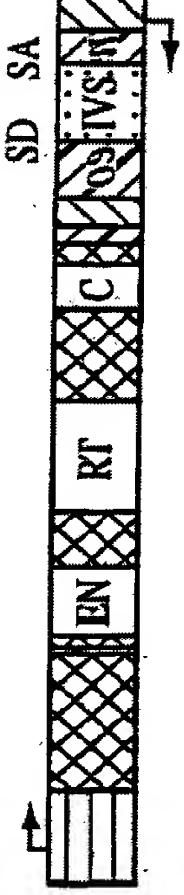



FIG. 2B-1

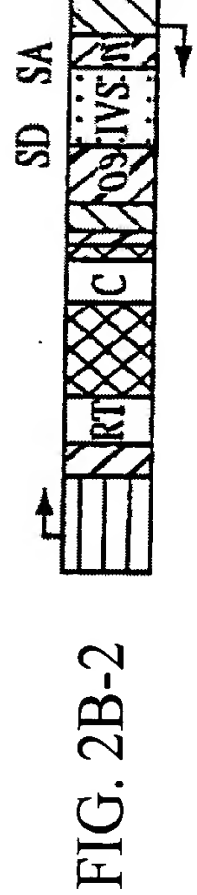



FIG. 2B-2

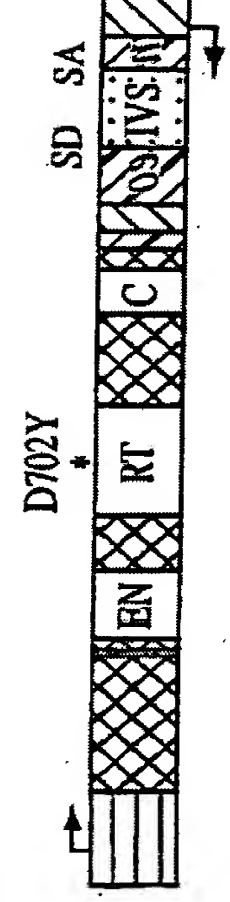



FIG. 2B-3

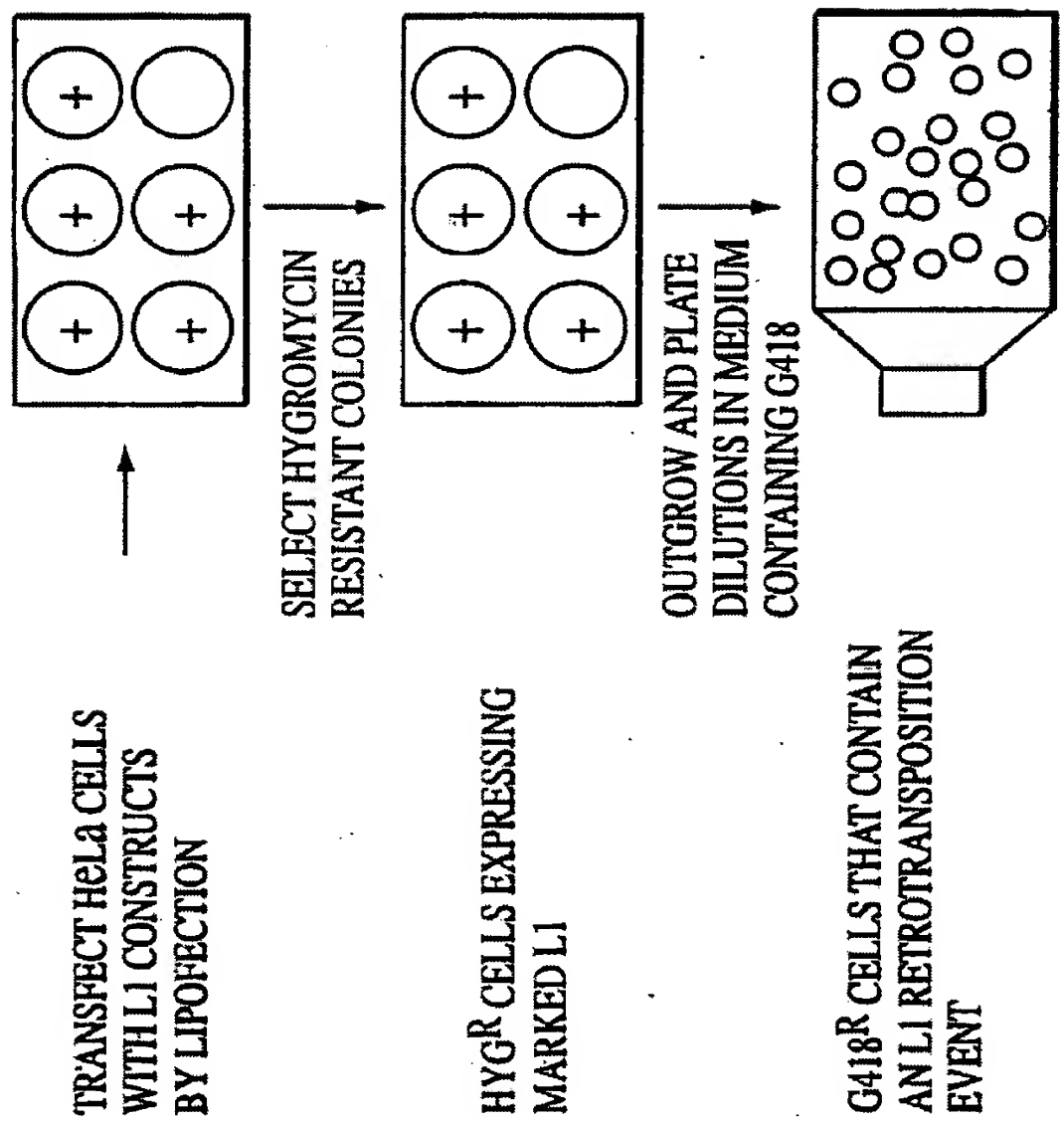

TRANSFECT HeLa CELLS
WITH L1 CONSTRUCTS
BY LIPOFECTION
SELECT HYGROMYCIN
RESISTANT COLONIES
HYG$^R$ CELLS EXPRESSING
MARKED L1
OUTGROW AND PLATE
DILUTIONS IN MEDIUM
CONTAINING G418
G418$^R$ CELLS THAT CONTAIN
AN L1 RETROTRANSPOSITION
EVENT

FIG. 3A

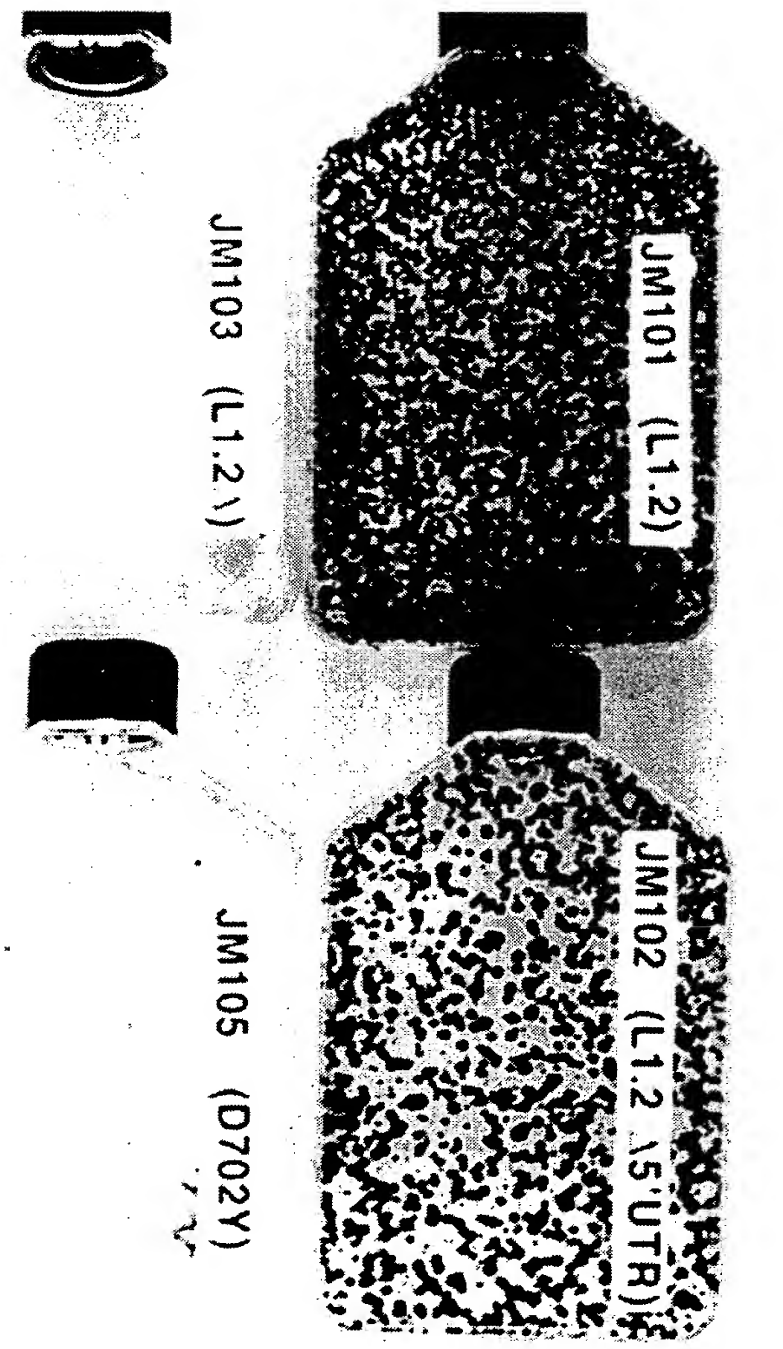



FIG. 3B

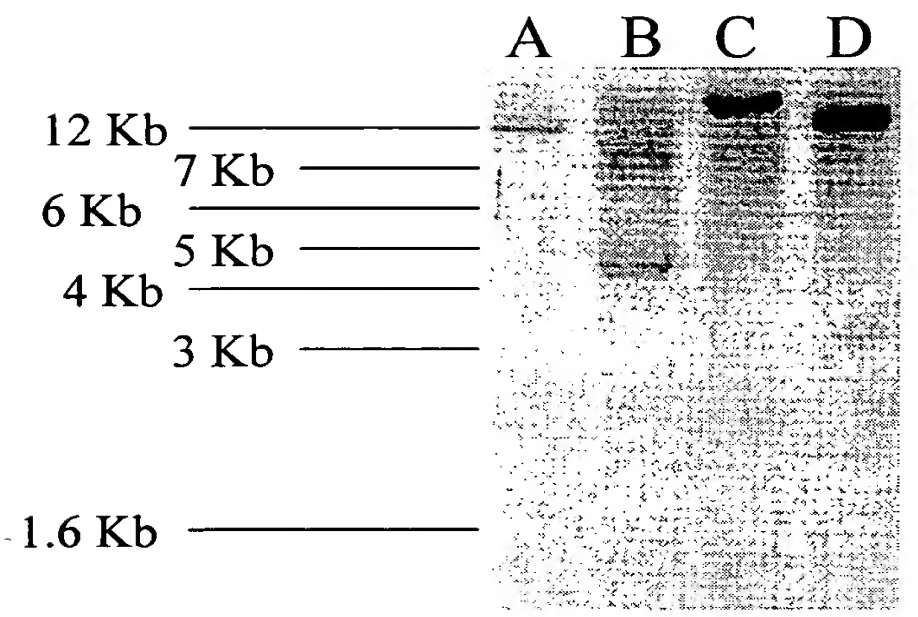



FIG. 4A

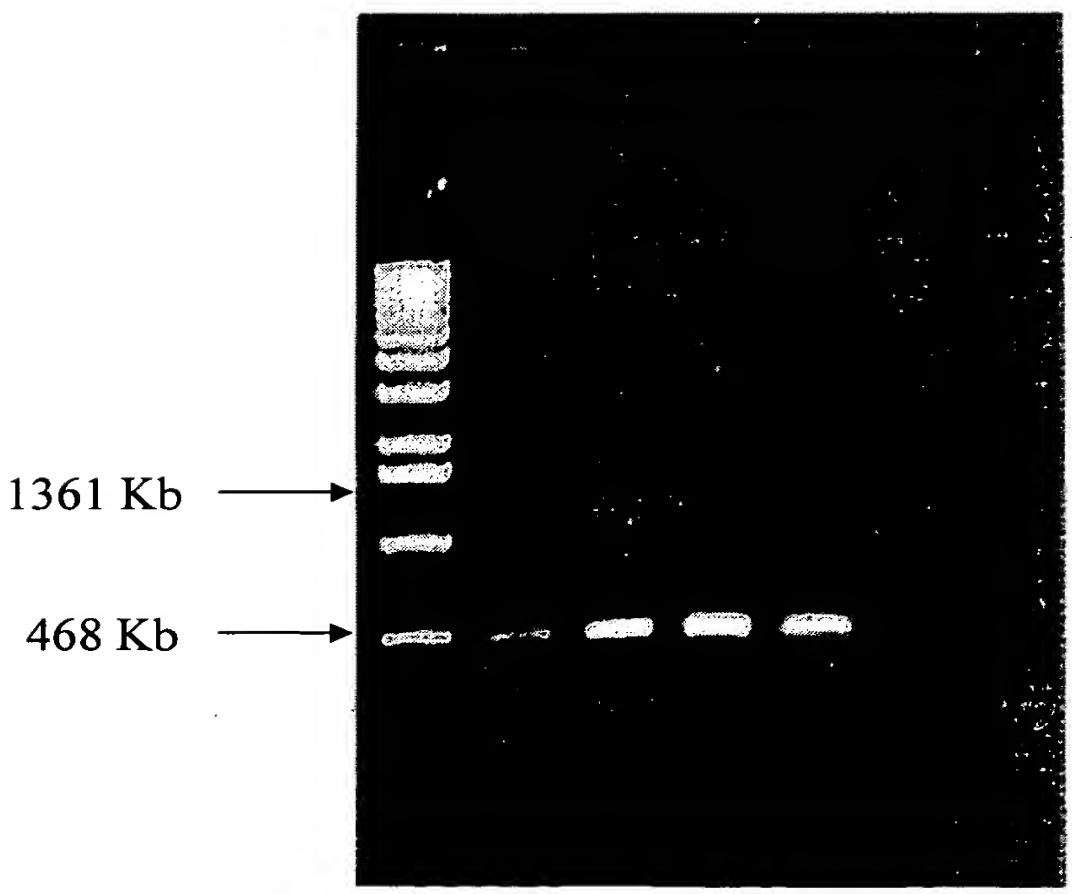



FIG. 4B-3

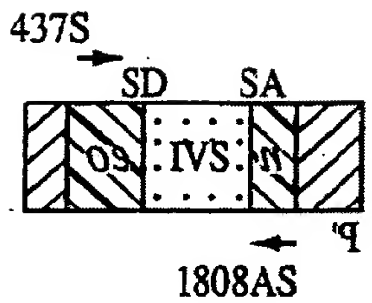



FIG. 4B-1

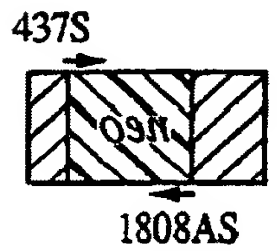



FIG. 4B-2

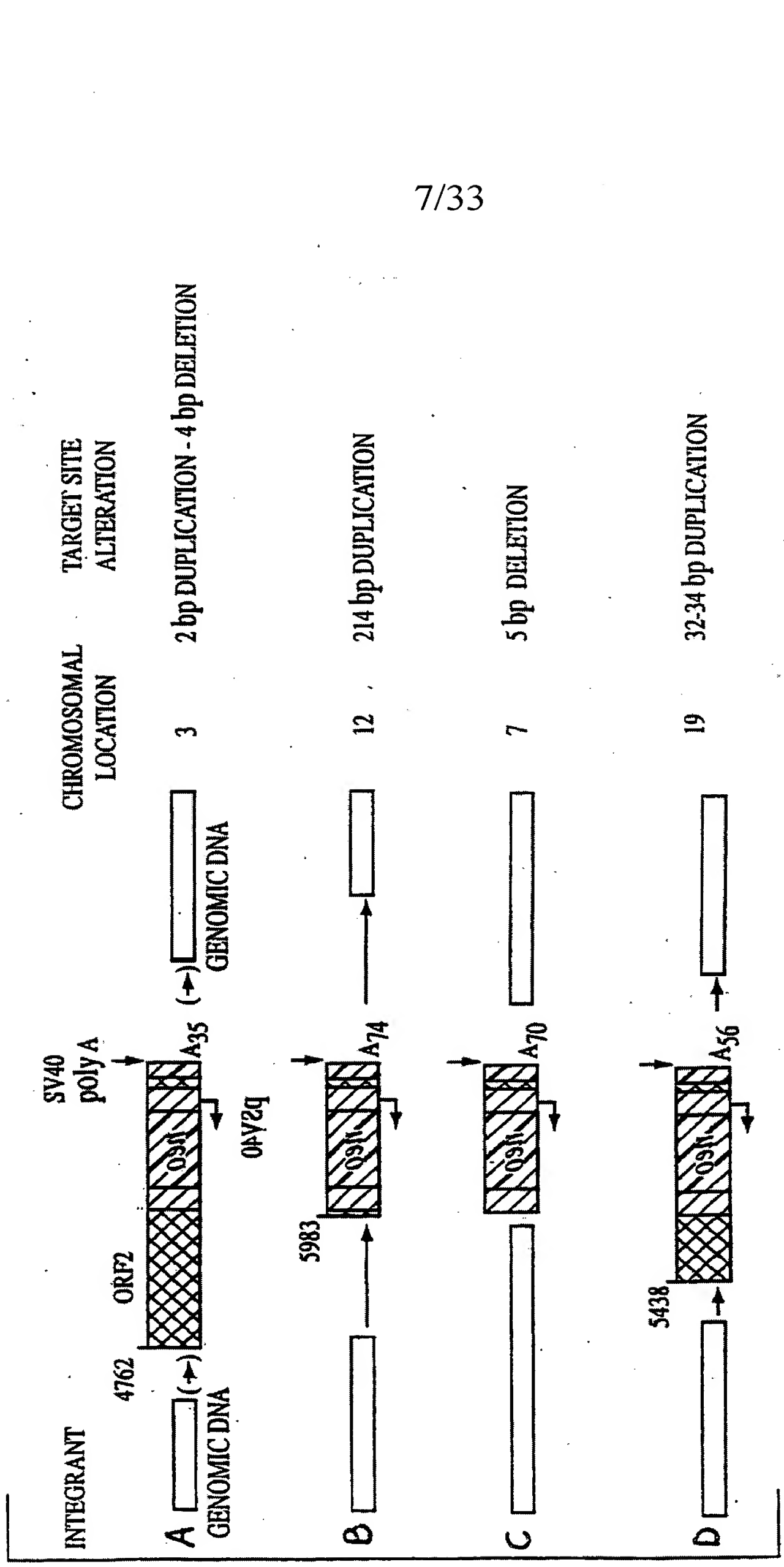

CHROMOSOMAL LOCATION
TARGET SITE ALTERATION
INTEGRANT
A
GENOMIC DNA
4762
ORF2
SV40 poly A
A35
3
2 bp DUPLICATION - 4 bp DELETION
B
5983
A74
12
214 bp DUPLICATION
C
A70
7
5 bp DELETION
D
5438
A56
19
32-34 bp DUPLICATION

FIG. 5

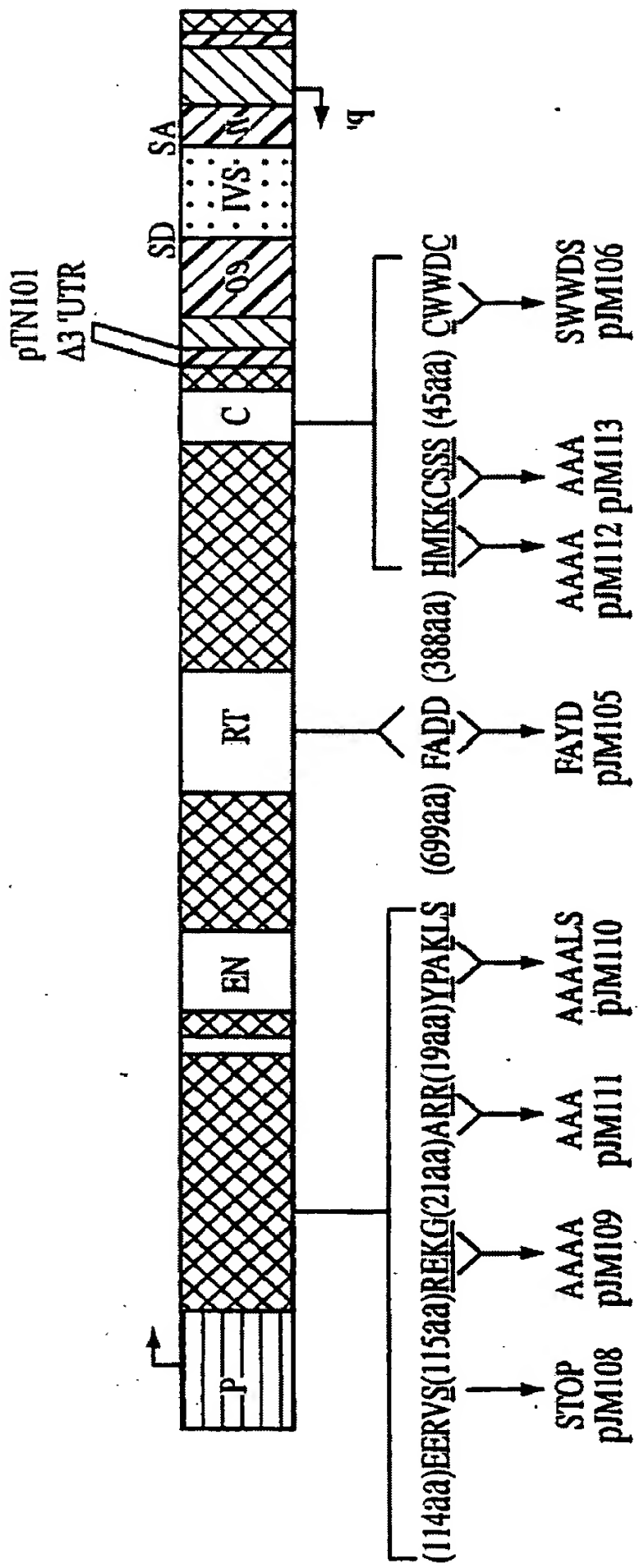

pTN101
Δ3'UTR
SD
SA
IVS
M
q
C
RT
EN
P
(388aa) HMKKCSSS (45aa) CWWDC
AAAA AAA
pJM112 pJM113
SWWDS
pJM106
(699aa) FADD (3
FAYD
pJM105
(114aa)EERVS(115aa)REKG(21aa)ARR(19aa)YPAKLS
STOP AAAA AAA AAAALS
pJM108 pJM109 pJM111 pJM110
ORF2 MUTANTS
ORF1 MUTANTS

FIG. 6

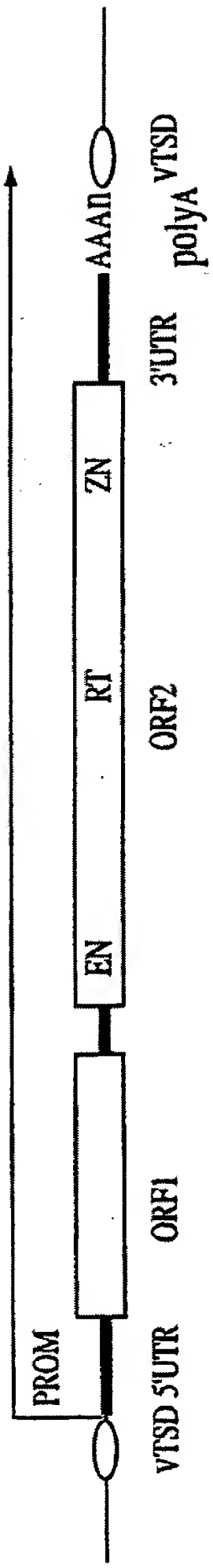



FIG. 7A

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | O | | O | | | | O |
| TAD | | .....MVQLKILYWNVGKS | (13) | YDIVAIQEPG | (22) | KGRAVIYVNK | (25) | PTTVYSIYSPILT | | | |
| L1Tc | (35) | DIEQNPGPIAVLQMNVSCL | (12) | ADIIAIQETW | (23) | GGGVAVLVRK | (31) | DLIVASAYMRPPP | | | |
| R1Bm | | ..MDIRPRLPIGQINLGGA | (15) | LDIVLVQEQY | (13) | KAGVYIRNRV | (22) | DLYMVSAYFQYSD | | | |
| FDm | | ....IMATLFIATWNANGV | (15) | IDVMLLSETH | (23) | HGGTAILIRN | (32) | LLTLAAVYCPPRF | | | |
| GDm | | ....MQISLNIVFWNANGL | (15) | IDILLVSESH | (24) | RGGAAMLIKS | (27) | DITVGAVYPRHEF | | | |
| JOCK | | ...MTQPTLKIGLWNARGL | (15) | IDVMLTTETH | (24) | RGGSAVIIKS | (27) | TVTVAAVYLPPAE | | | |
| IDm | | ......MSLTVIQWNLKGY | (15) | PHIISLQETH | (23) | FGGVRILVHK | (24) | KLNIFSTYISPTK | | | |
| L1Hs | | .MTGSNSHITILTLNINGL | (17) | PSVCCIQETH | (25) | KAGVAILVSD | (27) | ELTILNIYAPNTG | | | |
| Tx1 | | ......MALSISTLNTNGC | (17) | YSVSFLQETH | (25) | SCGVVTLFSD | (27) | TYNLMNVYAPTTG | | | |
| Cin4 | (10) | GYYPMNTNCCIFSWNVRGL | (17) | ATSVCLQETK | (27) | GASGGILIAC | (29) | VWDLTAVYGPQQE | | | |
| Dre | (04) | NKTIKKNTIRIGVWNVQGS | (17) | LDAALLTETN | (27) | QGVSQIIINT | (23) | QIKCTTIYAPAKS | | | |
| APHs | (53) | SPSGKPATLKICSWNVDGL | (16) | PDILCLQETK | (28) | GYSGVGLLSR | (27) | SFVLVTAYVPNAG | | | |
| Retrotransposons | | | 12-17 | | 13-27 | | 22-31 | | | 6-23 | |
| AP endonucleases | | | 15-17 | | 23-28 | | 27 | | | 27-28 | |
| DNase I | | .....MLKIAAFNIRTF | (20) | YDIVLIQEVR | | | (120) | | | | |
| L1Hs mutants | | | ↓ N14A | | ↓ E43A | | | | | | |

FIG. 7 | FIG. 7B-1 | FIG. 7B-2

FIG. 7B-1

```
                                                  O              O
          O O                      O              O             OOO
(23) NLVAVGDLNLHHPDWD (29) GE.PTRLGNATRGERDGTIDHAWLS (16) GSDHCPQEIWVQV
(17) PLLLCGDFNMHHPQWE (25) GE.ITTARGTRER...SCIDLTWSK (13) LSDHYVLTFTLHQ
(19) RVVICADTNAHSPLWH (35) GHLPTFSTANGE....SYVDVTLST (14) SSDHRLIVFGVGG
(16) HFIAAGDYNAKHTHWG (26) PGSPTYWPSDLN.KLPDLIDFAVTK (15) SSDHSPVLIHLRR
(16) RFIAAGDFNAKHSWWG (24) TGEPTHWPSDPS.KQPDLLDIAICK (15) VSDHSAVNLLLNI
(16) KFIAGGDYNAKHAWWG (24) TGEPTFYSYNPL.LTPSALDFFITC (15) SSDHLPILAVLHA
(16) PSLITGDFNGWHPSWG (24) DKSPTHFSTH...NTYSHIDLTLCS (16) GSDHFPIITTLFP
(18) HTLIMGDFNTPLSTLD (34) TE.YTF..FSAPHHTYSKIDHIVGS (16) LSDHSAIKLELRI
(21) ALIIGGDFNYTLDARD (34) VA.FTYVRVRDGHVSQSRIDRIYIS (16) FSDHNCVSLRMSI
(19) EWLILGDFNMIRRVGE (30) KK.FT.WSNEQDDPTMSRIDRLMAT (18) TSDHSPLLMQGHS
(17) SDIITGDFNVDCSVDN (19) NG.ITFPR.....NKSTIDRVFVS (17) KSDHNMVIIELKI
(27) PLVLCGDLNVAHEEID (45) TF.WTYMMNARSKNVGWRLDYFLLS (17) GSDHCPITLYLAL

                    21-35                          13-18

                    44-50                          17-21

DVMLMGDFNADCSYVT (31) ................CAYDRIVVA (31) ISDHYPVEVTLT
      ↓                                    ↓              ↓
   D145A                                D205G          H230A
```

FIG. 7B-2

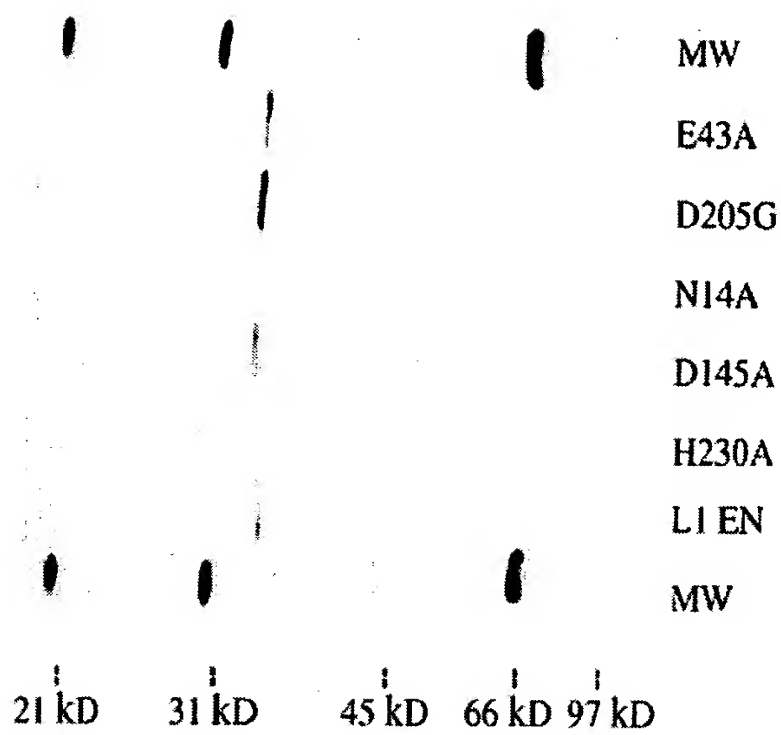

MW
E43A
D205G
N14A
D145A
H230A
L1 EN
MW
21 kD
31 kD
45 kD
66 kD
97 kD

FIG. 8A

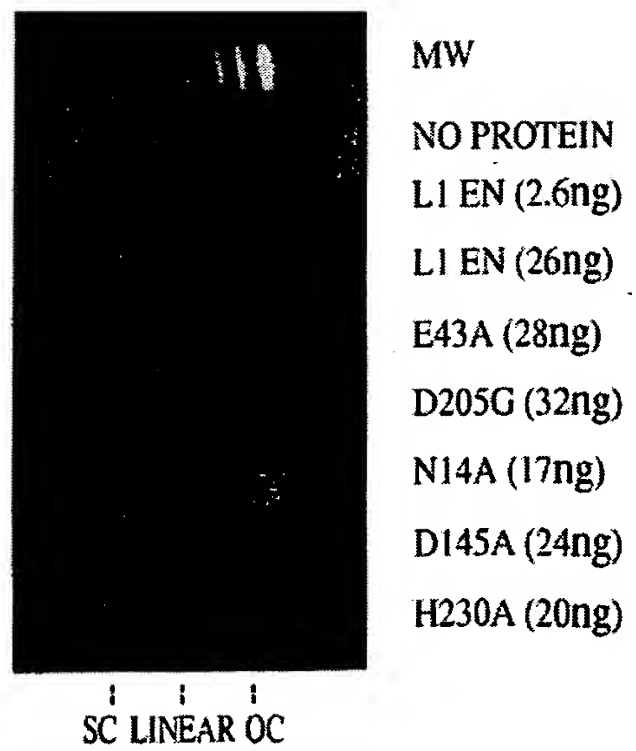

MW
NO PROTEIN
L1 EN (2.6ng)
L1 EN (26ng)
E43A (28ng)
D205G (32ng)
N14A (17ng)
D145A (24ng)
H230A (20ng)
SC
LINEAR
OC

FIG. 8B

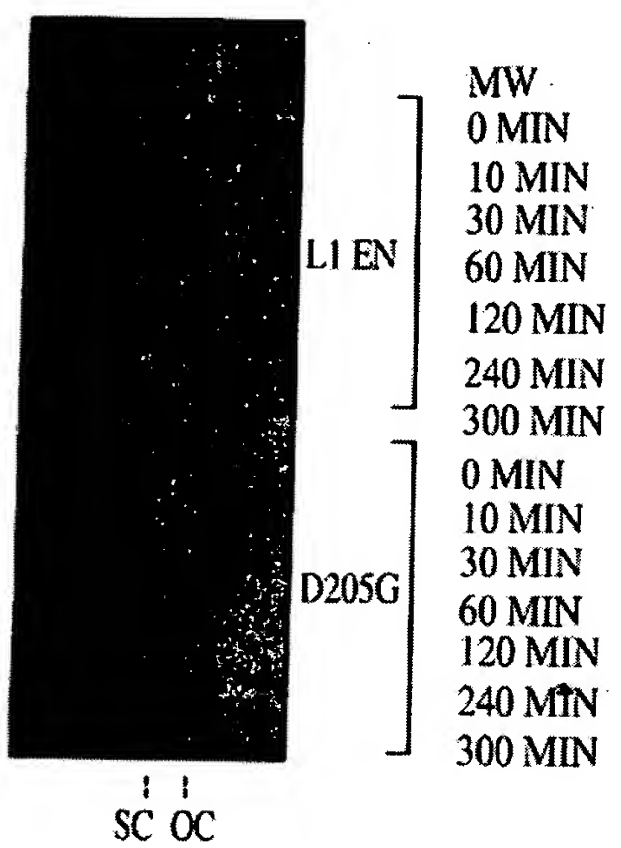

MW
0 MIN
10 MIN
30 MIN
60 MIN
120 MIN
240 MIN
300 MIN
L1 EN
0 MIN
10 MIN
30 MIN
60 MIN
120 MIN
240 MIN
300 MIN
D205G
SC
OC

FIG. 8C

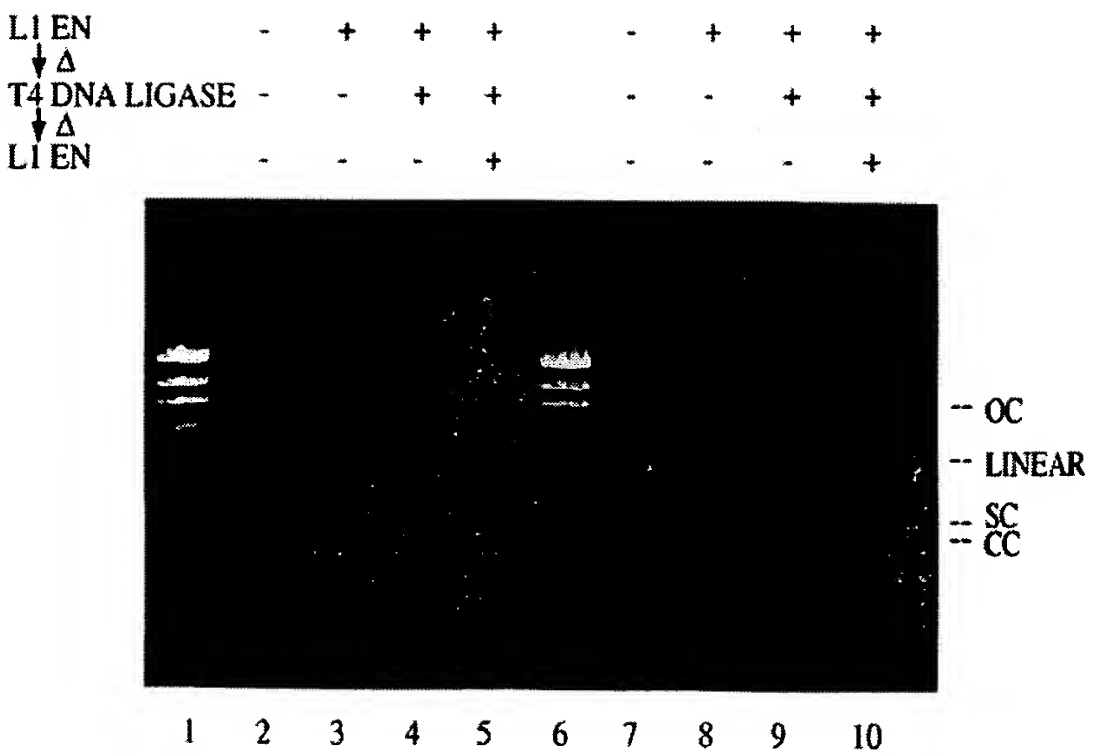

L1 EN - + + + - + + +
Δ
T4 DNA LIGASE - - + + - - + +
Δ
L1 EN - - - + - - - +
-- OC
-- LINEAR
-- SC
-- CC
1 2 3 4 5 6 7 8 9 10

FIG. 9

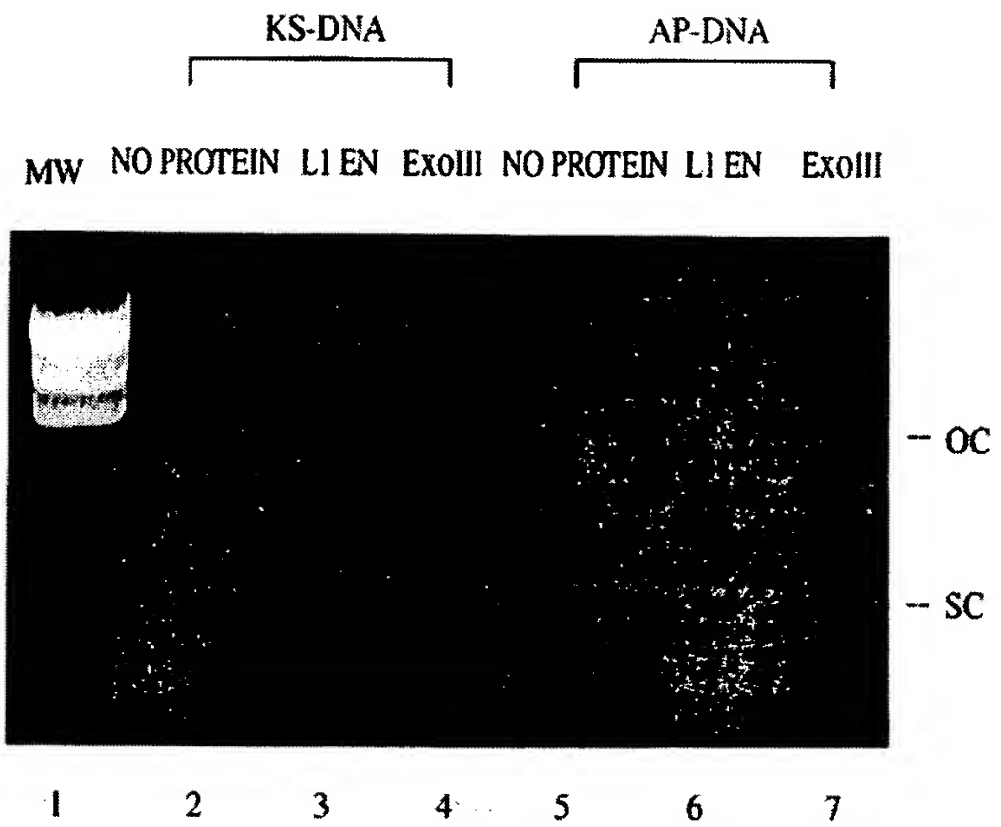



FIG. 10

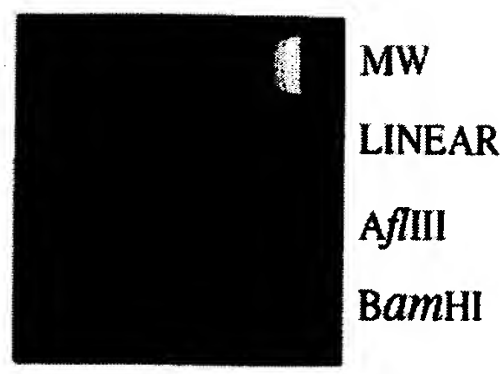



FIG. 11A

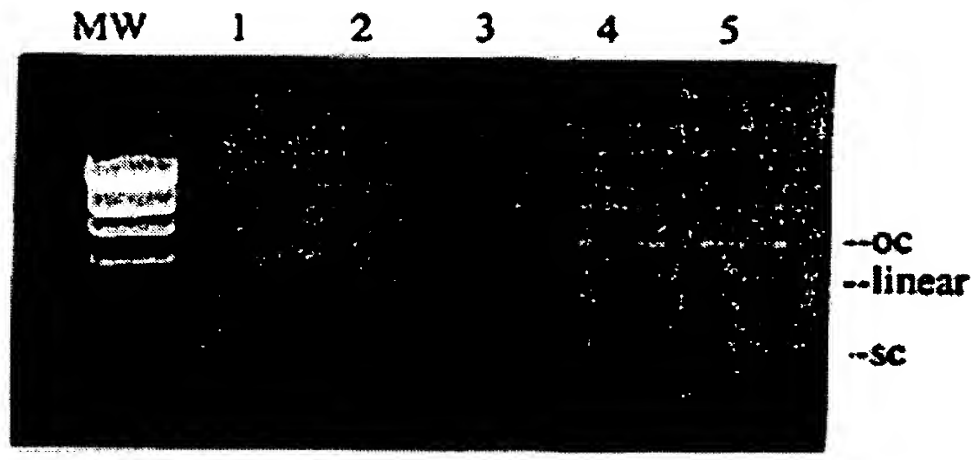



FIG. 11B

FIG. 11C-1


FIG. 11C-2


FIG. 11C-3

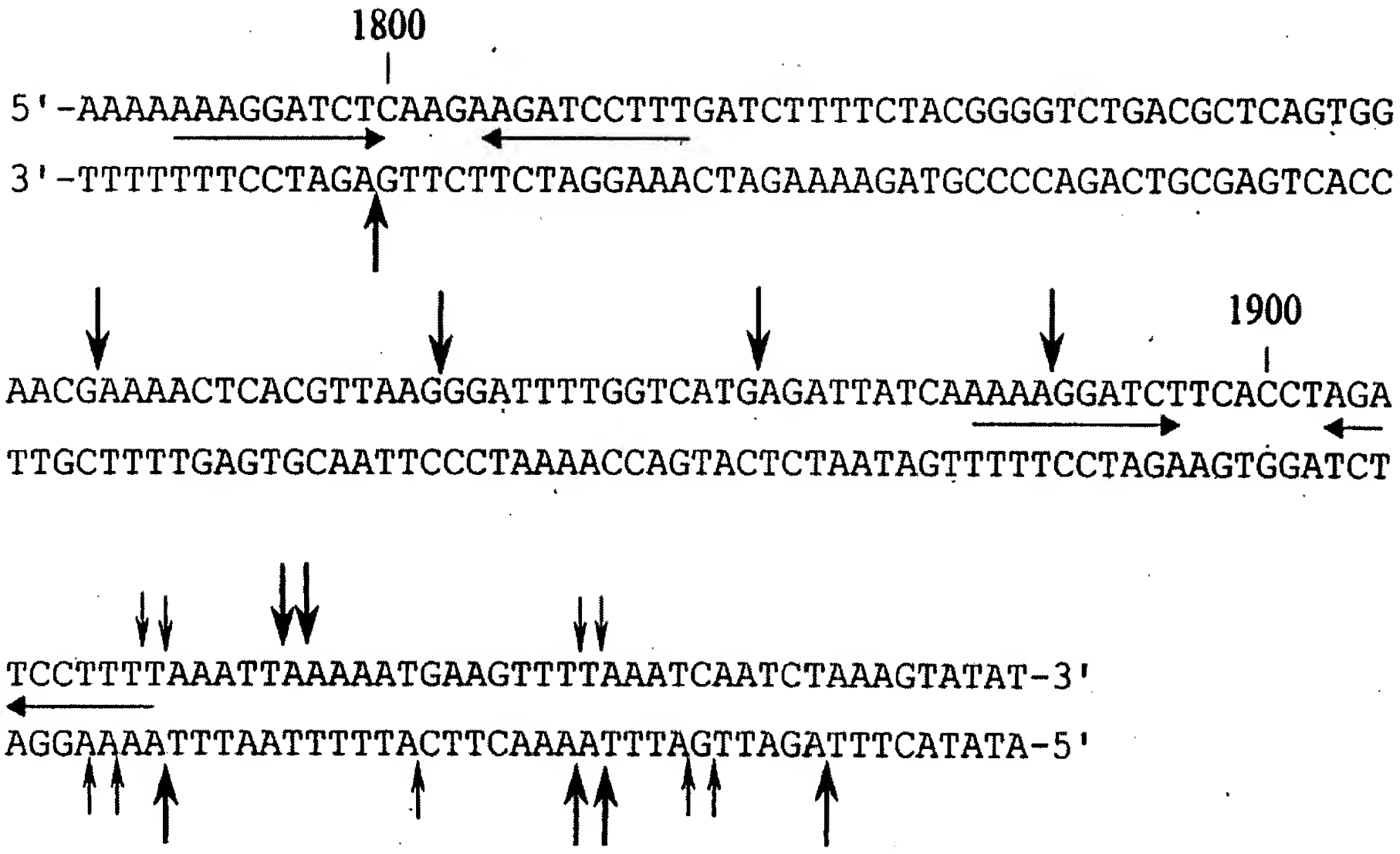

1800
5'-AAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGG
3'-TTTTTTTCCTAGAGTTCTTCTAGGAAACTAGAAAAGATGCCCCAGACTGCGAGTCACC
1900
AACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGA
TTGCTTTTGAGTGCAATTCCCTAAAACCAGTACTCTAATAGTTTTTCCTAGAAGTGGATCT
TCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATAT-3'
AGGAAAATTTAATTTTTACTTCAAAATTTAGTTAGATTTCATATA-5'

FIG. 11D

FIG. 12A

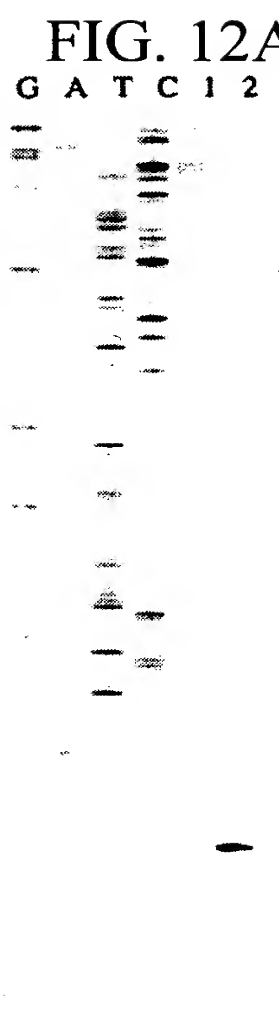



FIG. 12B

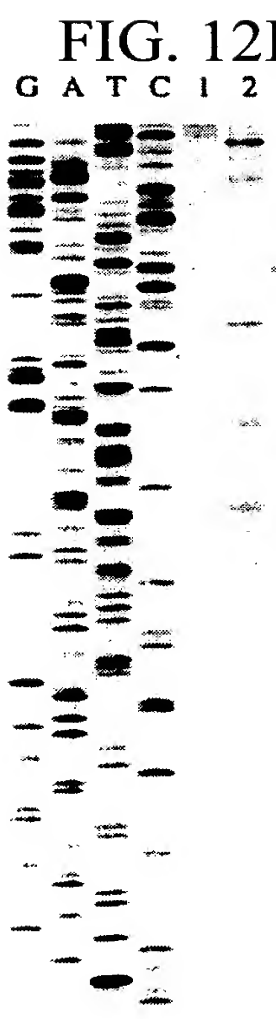
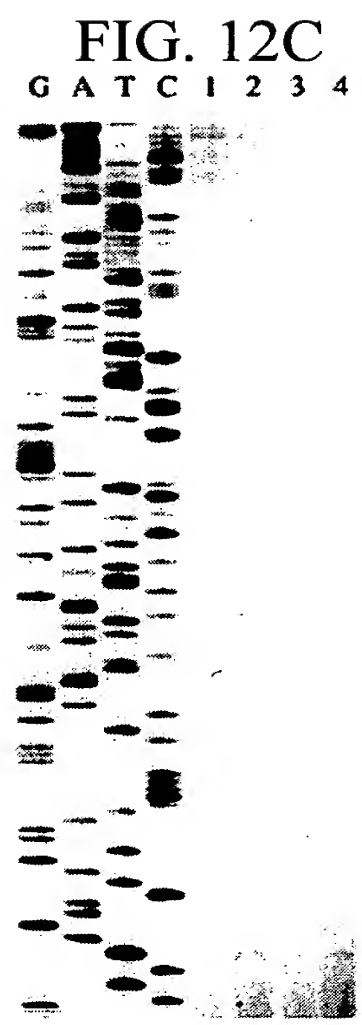



FIG. 12C



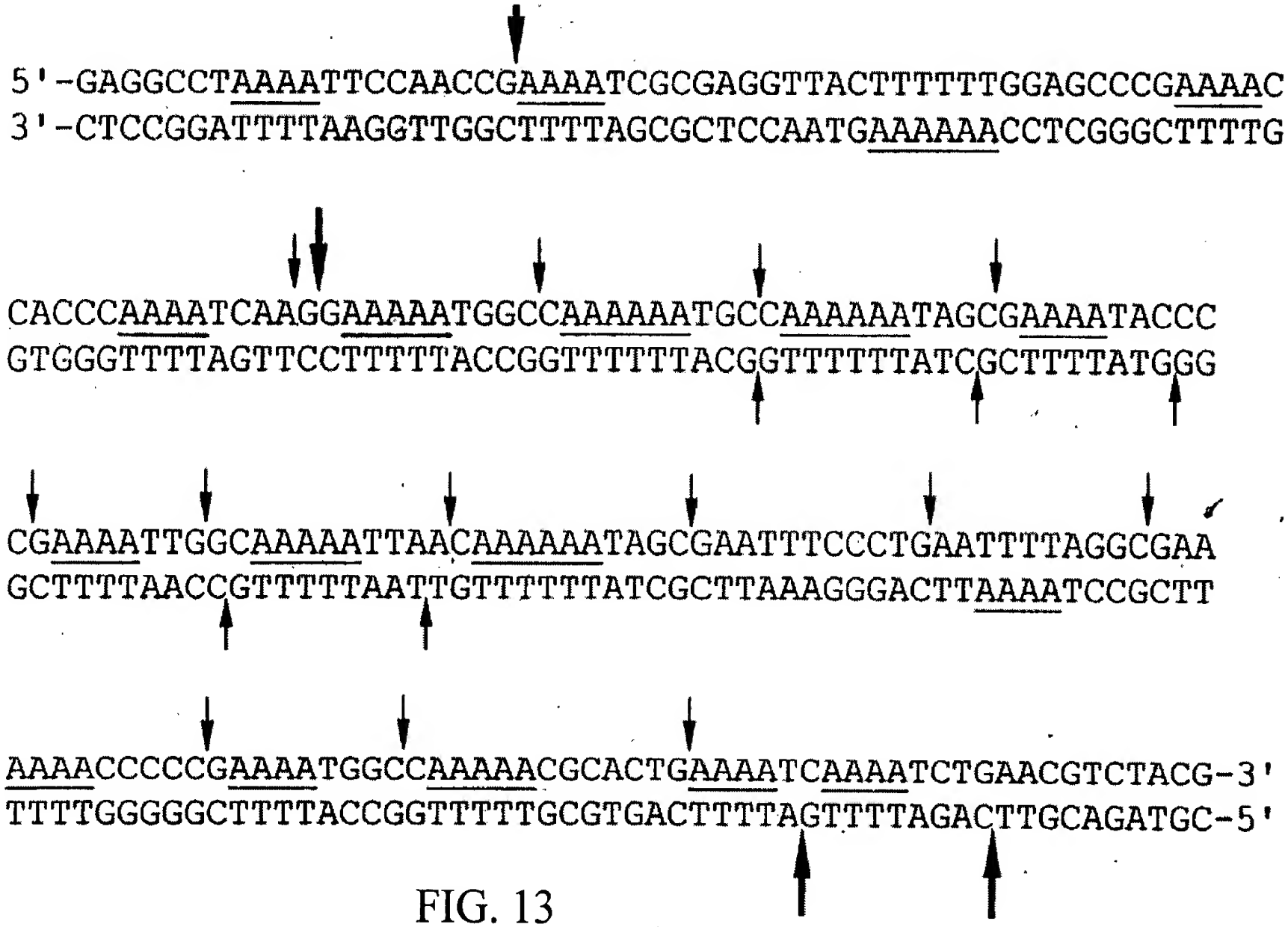

5'-GAGGCCTAAAATTCCAACCGAAAATCGCGAGGTTACTTTTTTGGAGCCCGAAAAC
3'-CTCCGGATTTTAAGGTTGGCTTTTAGCGCTCCAATGAAAAAACCTCGGGCTTTTG
CACCCAAAATCAAGGAAAAATGGCCAAAAAATGCCAAAAAATAGCGAAAATACCC
GTGGGTTTTAGTTCCTTTTTTACCGGTTTTTTTACGGTTTTTTTATCGCTTTTATGGG
CGAAAATTGGCAAAAATTAACAAAAAATAGCGAATTTCCCTGAATTTTAGGCGAA
GCTTTTAACCGTTTTTTAATTGTTTTTTTATCGCTTAAAGGGACTTAAAATCCGCTT
AAAACCCCCCGAAAATGGCCAAAAACGCACTGAAAATCAAAATCTGAACGTCTACG-3'
TTTTGGGGGCTTTTACCGGTTTTTGCGTGACTTTTAGTTTTAGACTTGCAGATGC-5'

FIG. 13

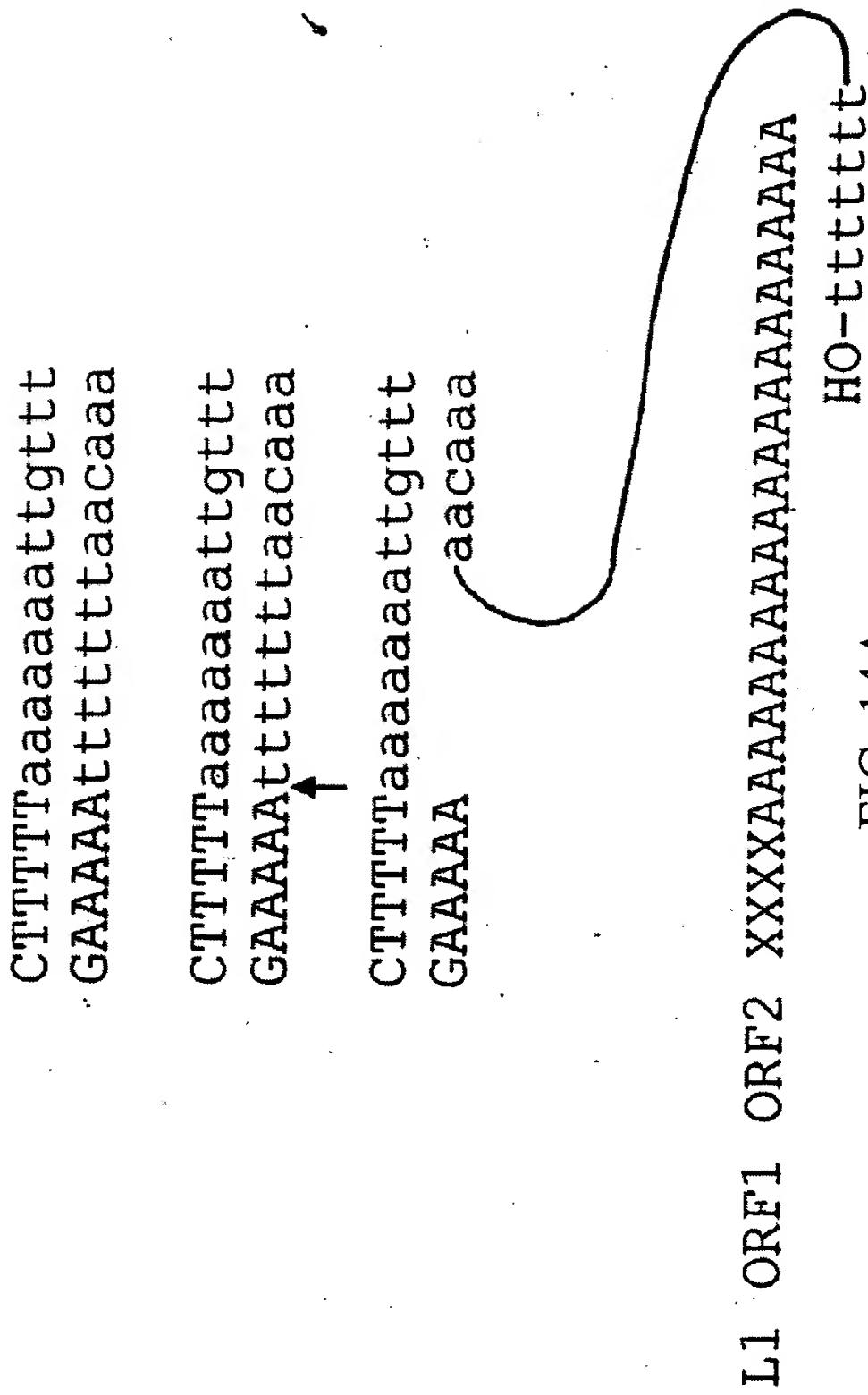

CTTTTaaaaaattg
GAAAAttttttaac
CTTTTaaaaaattg
GAAAAttttttaac
CTTTTaaaaaattg
GAAAAA
aac
L1 ORF1 ORF2 XXXXAAAAAAAAAA
HO-ttttttt

FIG. 14A

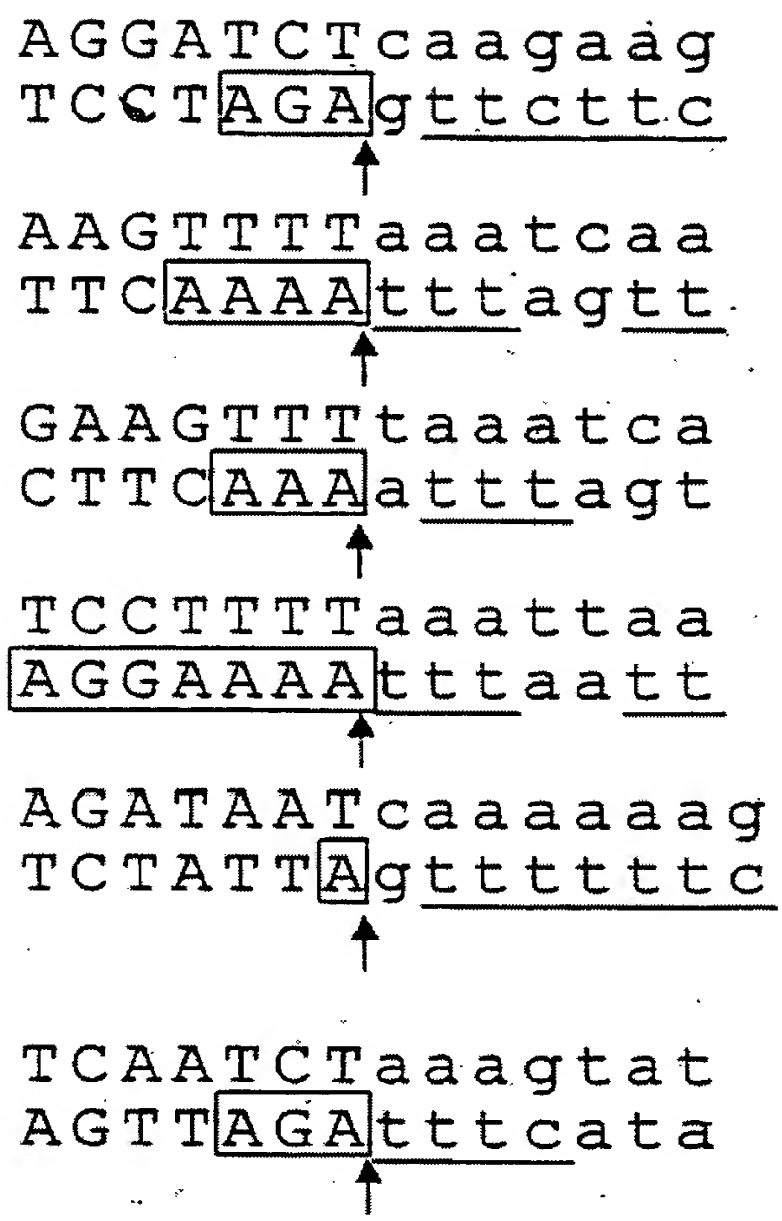

AGGATCTcaagaag
TCCTAGAgttcttc
AAGTTTTaaatcaa
TTCAAAAtttagtt
GAAGTTTtaaatca
CTTCAAAatttagt
TCCTTTTaaattaa
AGGAAAAtttaatt
AGATAATcaaaaaag
TCTATTAgttttttc
TCAATCTaaagtat
AGTTAGAtttcata

FIG. 14B-2

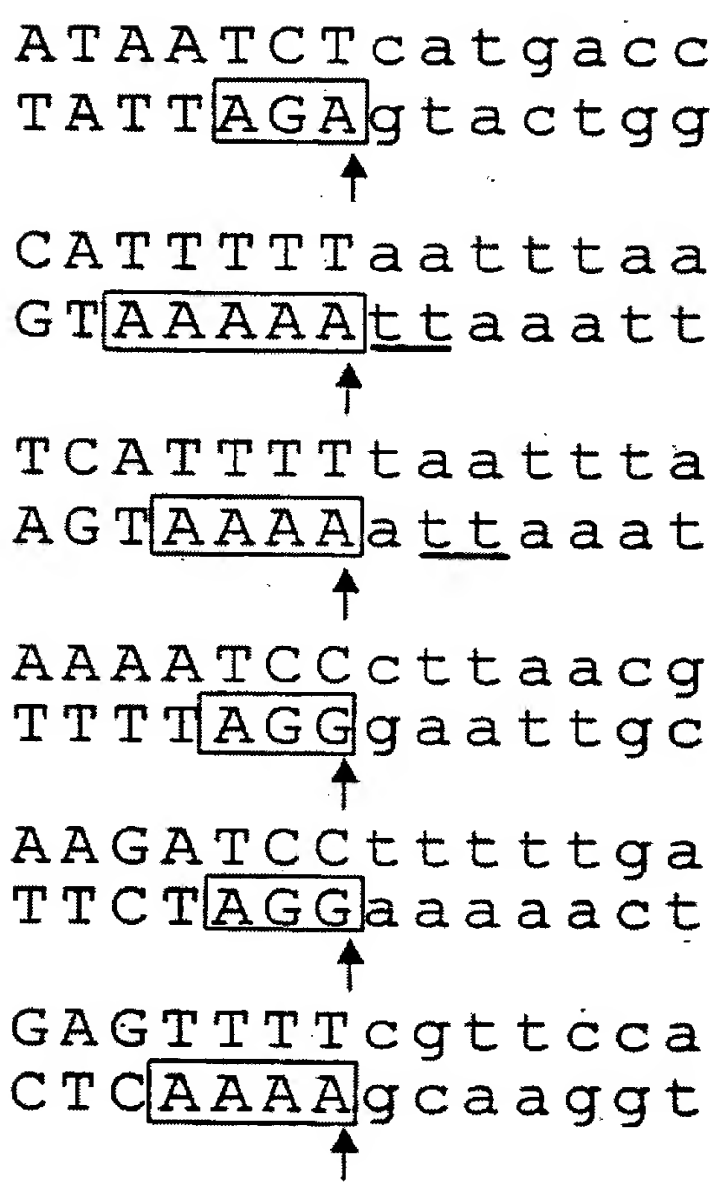

ATAATCTcatgacc
TATTAGAgtactgg
CATTTTTaatttaa
GTAAAAAttaaatt
TCATTTTtaatttta
AGTAAAAattaaat
AAAATCCcttaacg
TTTTAGGgaattgc
AAGATCCttttttga
TTCTAGGaaaaaact
GAGTTTTcgttcca
CTCAAAAgcaaggt

FIG. 14B-1

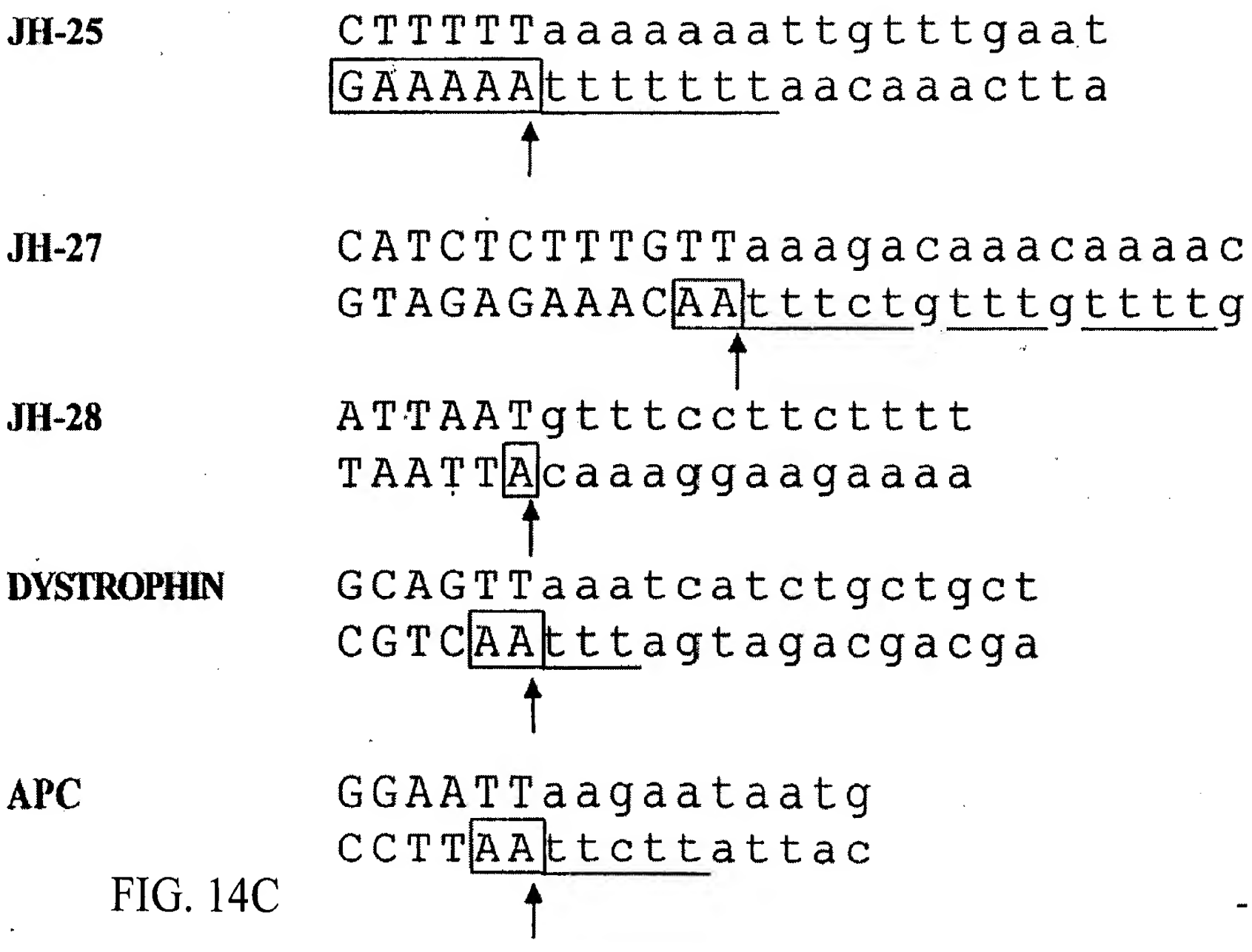
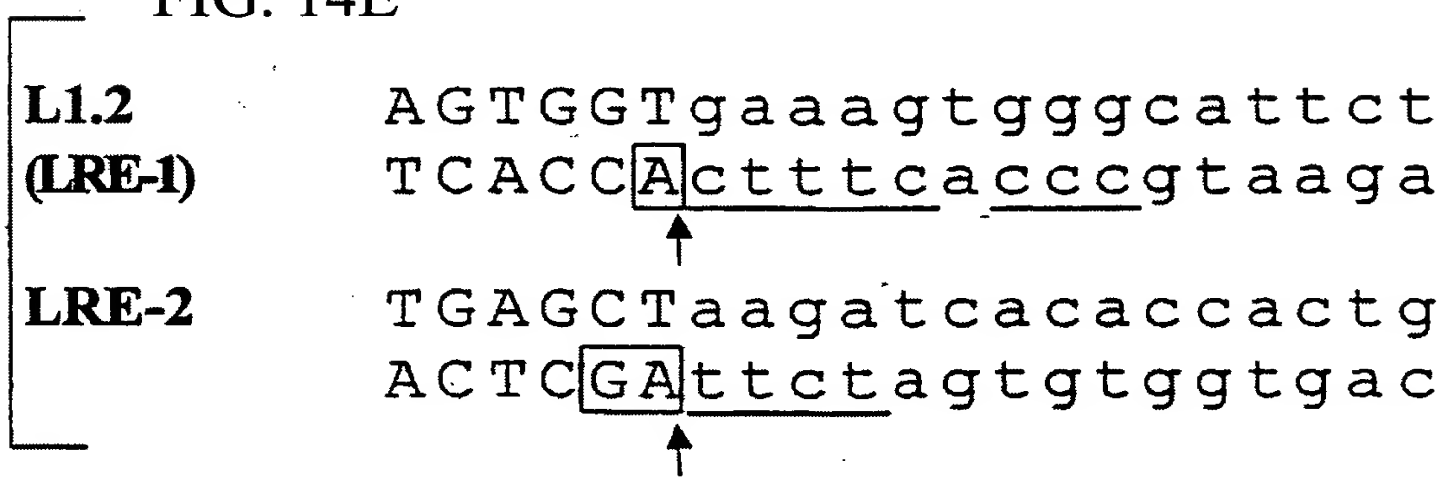

JH-25
CTTTTTaaaaaaaattgtttgaat
GAAAAAttttttttaacaaactta
JH-27
CATCTCTTTGTTaaagacaaacaaaac
GTAGAGAAACAAtttctgtttgttttg
JH-28
ATTAATgtttccttctttt
TAATTAcaaaggaagaaaa
DYSTROPHIN
GCAGTTaaatcatctgctgct
CGTCAAtttagtagacgacga
APC
GGAATTaagaataatg
CCTTAAttcttattac

FIG. 14C

FIG. 14D


FIG. 14E



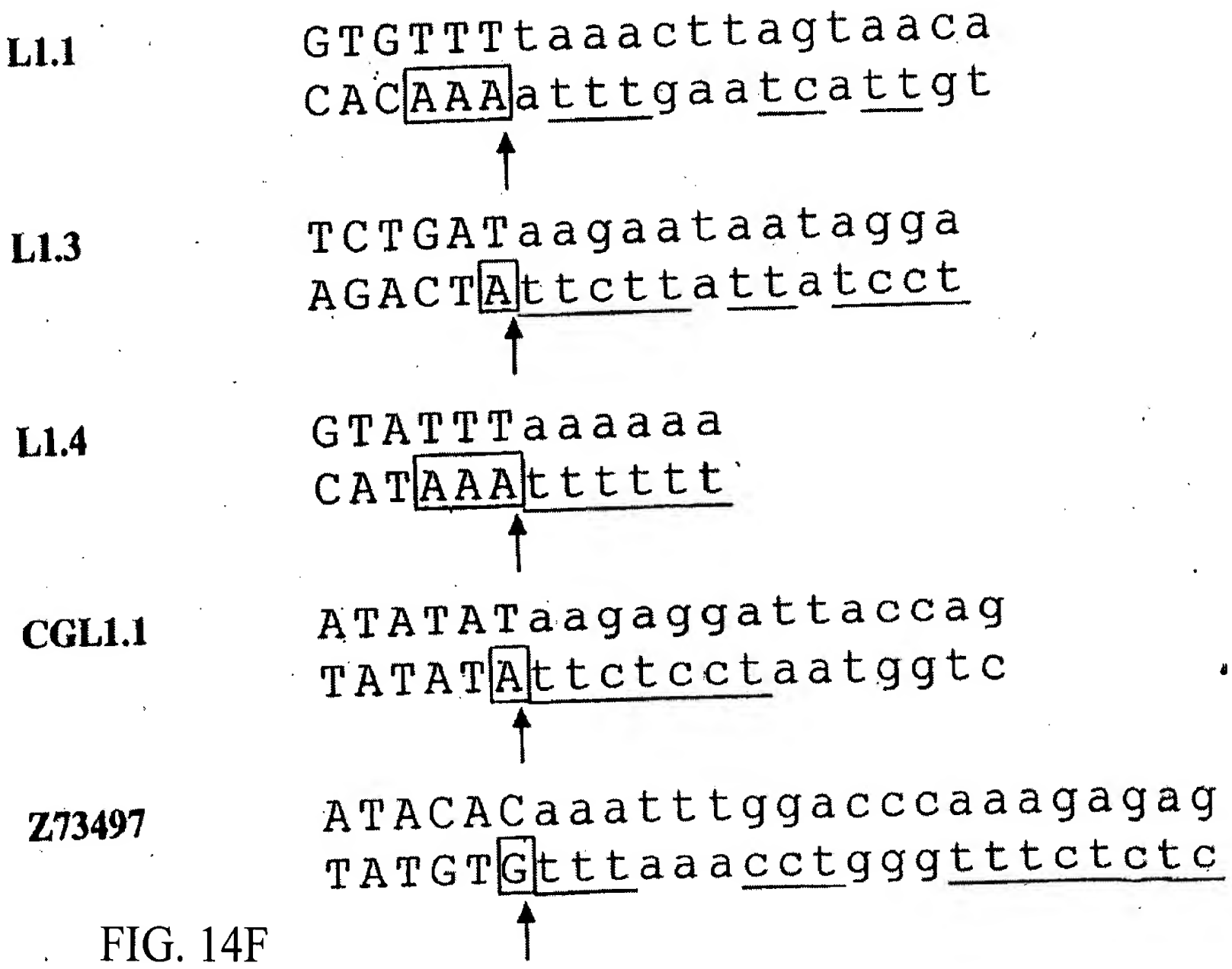
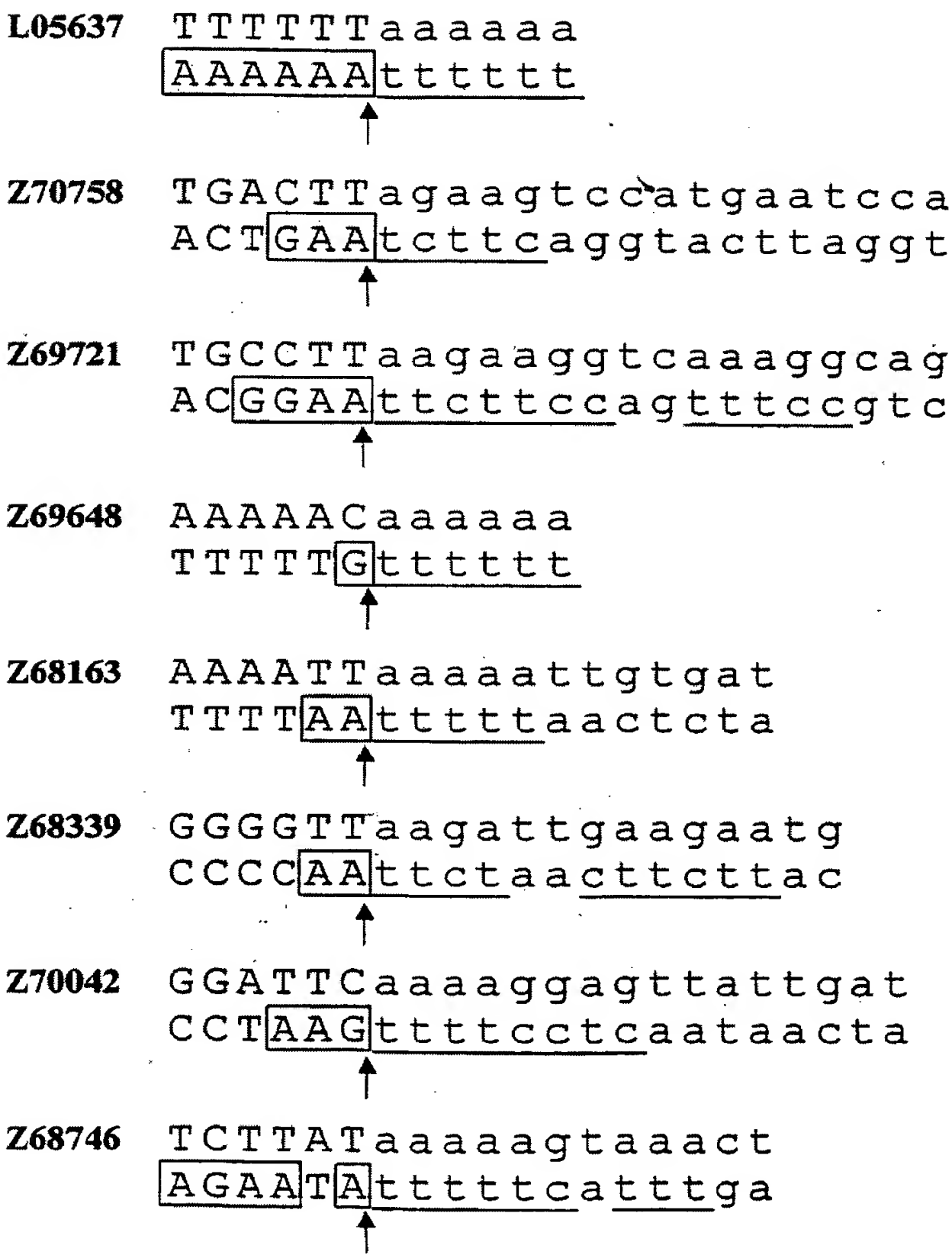

L1.1
GTGTTTtaaacttagtaaca
CACAAAatttgaatcattgt
L1.3
TCTGATaagaataatagga
AGACTAttcttattatcct
L1.4
GTATTTaaaaaa
CATAAAttttttt
CGL1.1
ATATATaagaggattaccag
TATATAttctcctaatggtc
Z73497
ATACACaaatttggacccaaagagag
TATGTGtttaaacctgggtttctctc

FIG. 14F

FIG. 14G


L05637
TTTTTTaaaaaa
AAAAAAttttttt
Z70758
TGACTTagaagtccatgaatcca
ACTGAAtcttcaggtacttaggt
Z69721
TGCCTTaagaaggtcaaaggcag
ACGGAAttcttccagtttccgtc
Z69648
AAAAACaaaaaa
TTTTTGttttttt
Z68163
AAAATTaaaaattgtgat
TTTTAAttttttaactcta
Z68339
GGGGTTaagattgaagaatg
CCCCAAttctaacttcttac
Z70042
GGATTCaaaaggagttattgat
CCTAAGttttcctcaataacta
Z68746
TCTTATaaaaagtaaact
AGAATAttttttcatttga

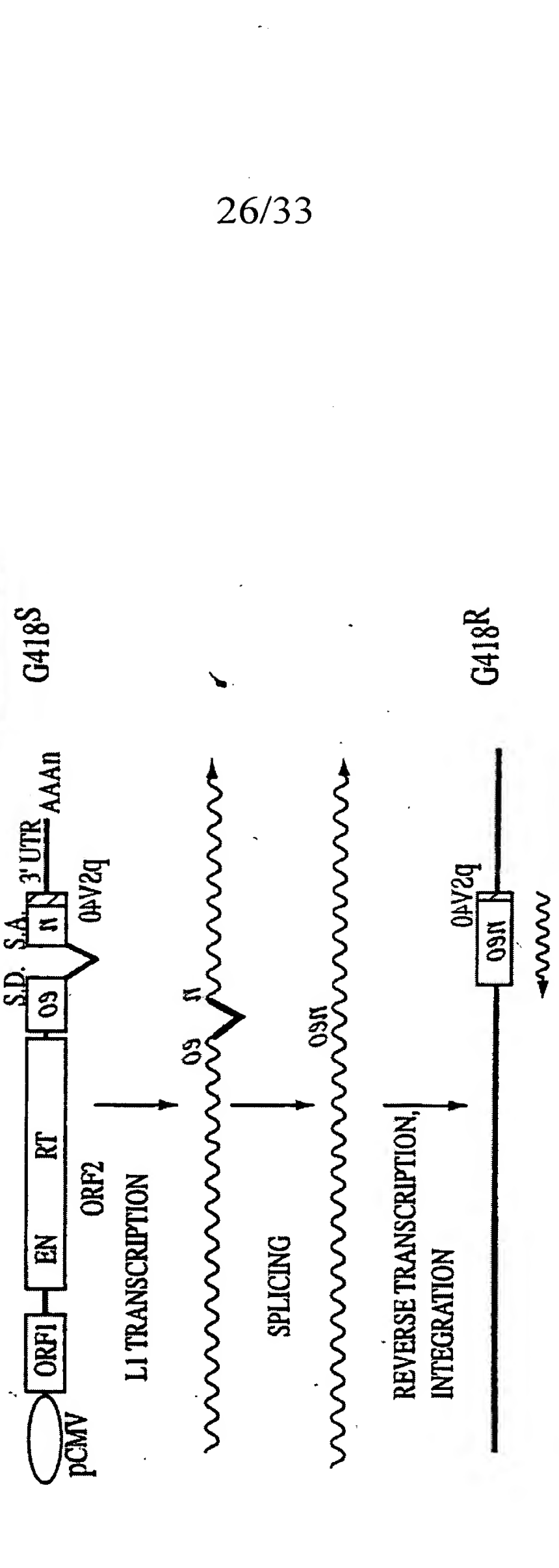

PHENOTYPE
G418S
G418R
pCMV
ORF1
EN
RT
ORF2
S.D.
S.A.
3'UTR
AAAn
L1 TRANSCRIPTION
SPLICING
REVERSE TRANSCRIPTION,
INTEGRATION

FIG. 15A

| CONSTRUCT | | TRANSPOSITION FREQUENCY ($10^{-6}$ cell$^{-1}$) |
|---|---|---|
| WILDTYPE L1 | | 335 |
| D703Y | ($RT^-$) | 0.5 |
| N14 | ($EN^-$) | 3.4 |
| D145A | ($EN^-$) | 1.0 |
| D205G | ($EN^-$) | 0.7 |
| H230A | ($EN^-$) | 1.3 |

FIG. 15B

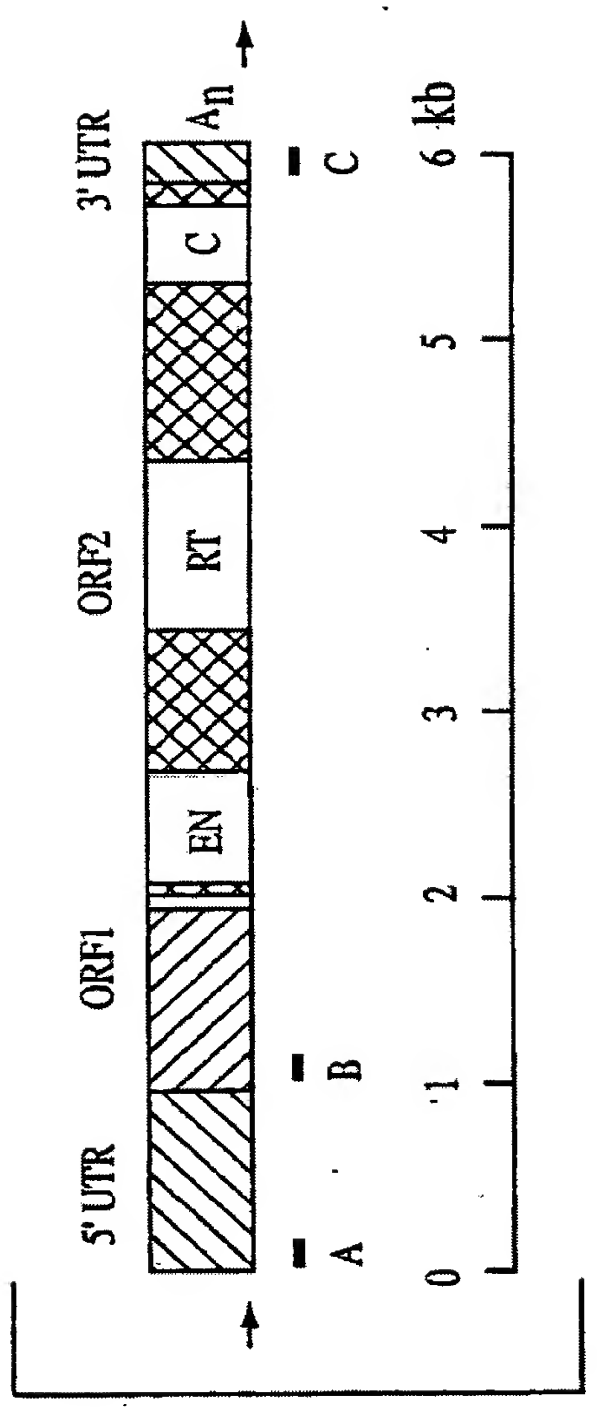



FIG. 16

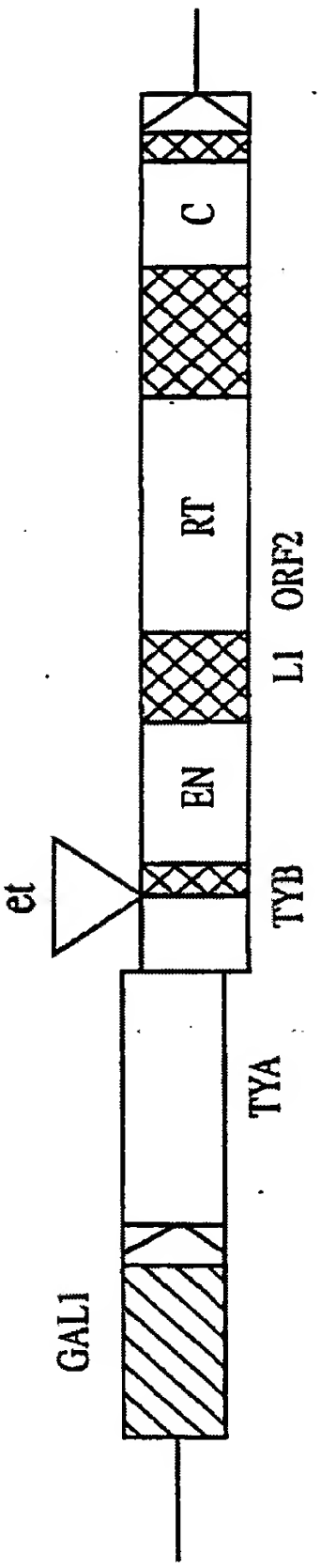



FIG. 17A

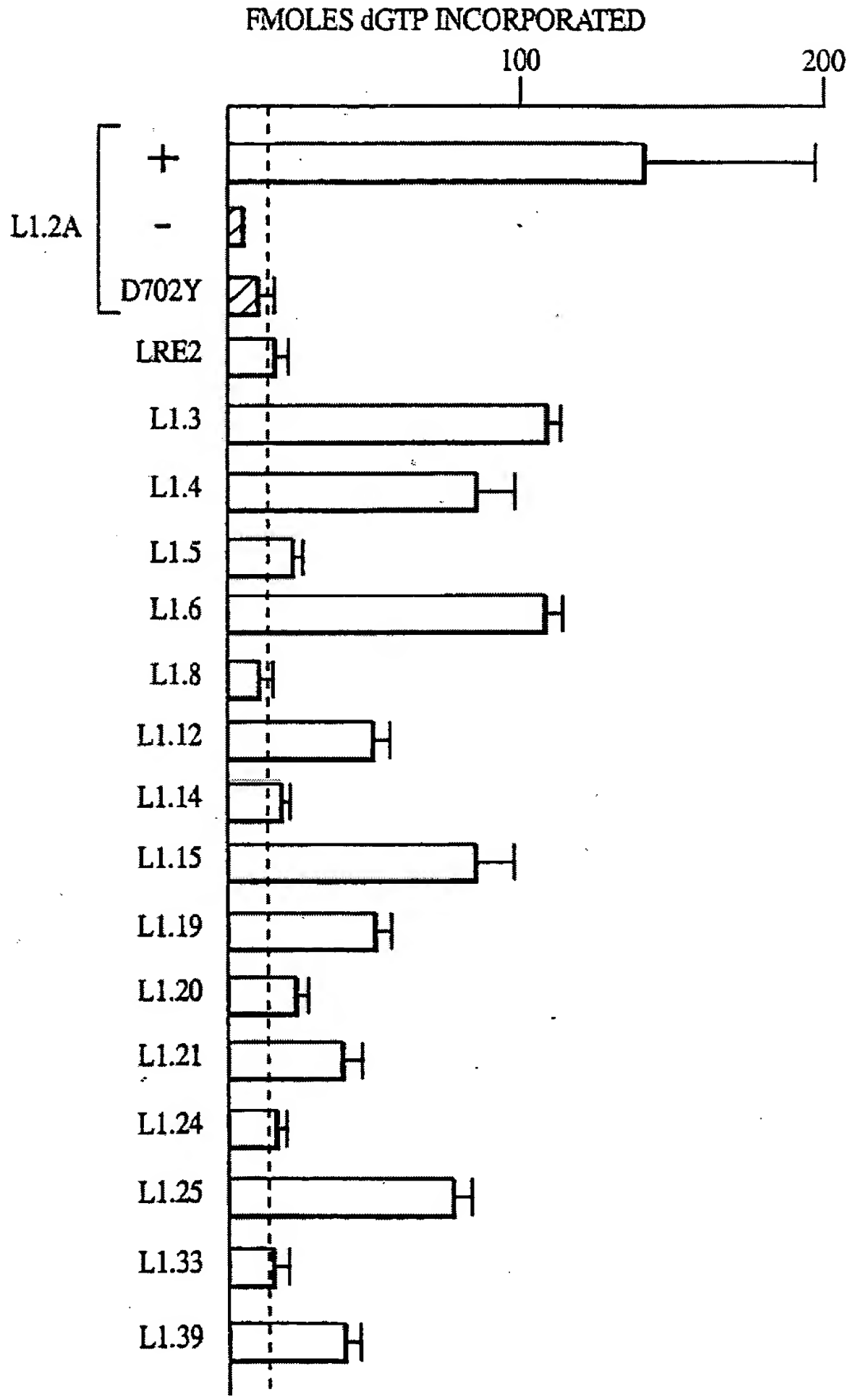

FMOLES dGTP INCORPORATED
100
200
L1.2A
+
-
D702Y
LRE2
L1.3
L1.4
L1.5
L1.6
L1.8
L1.12
L1.14
L1.15
L1.19
L1.20
L1.21
L1.24
L1.25
L1.33
L1.39

FIG. 17B

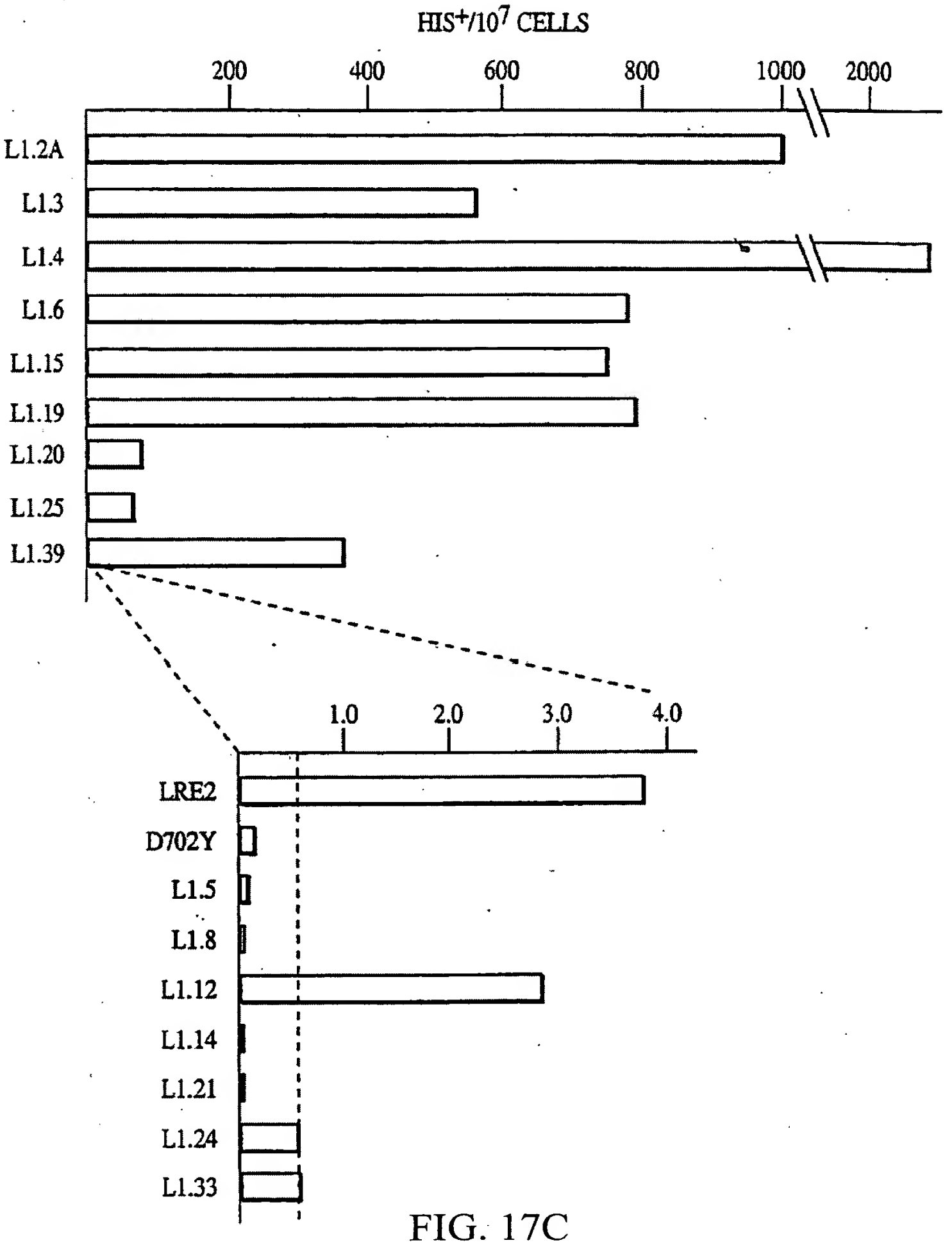

HIS+/10^7 CELLS
200
400
600
800
1000
2000
L1.2A
L1.3
L1.4
L1.6
L1.15
L1.19
L1.20
L1.25
L1.39
1.0
2.0
3.0
4.0
LRE2
D702Y
L1.5
L1.8
L1.12
L1.14
L1.21
L1.24
L1.33

FIG. 17C

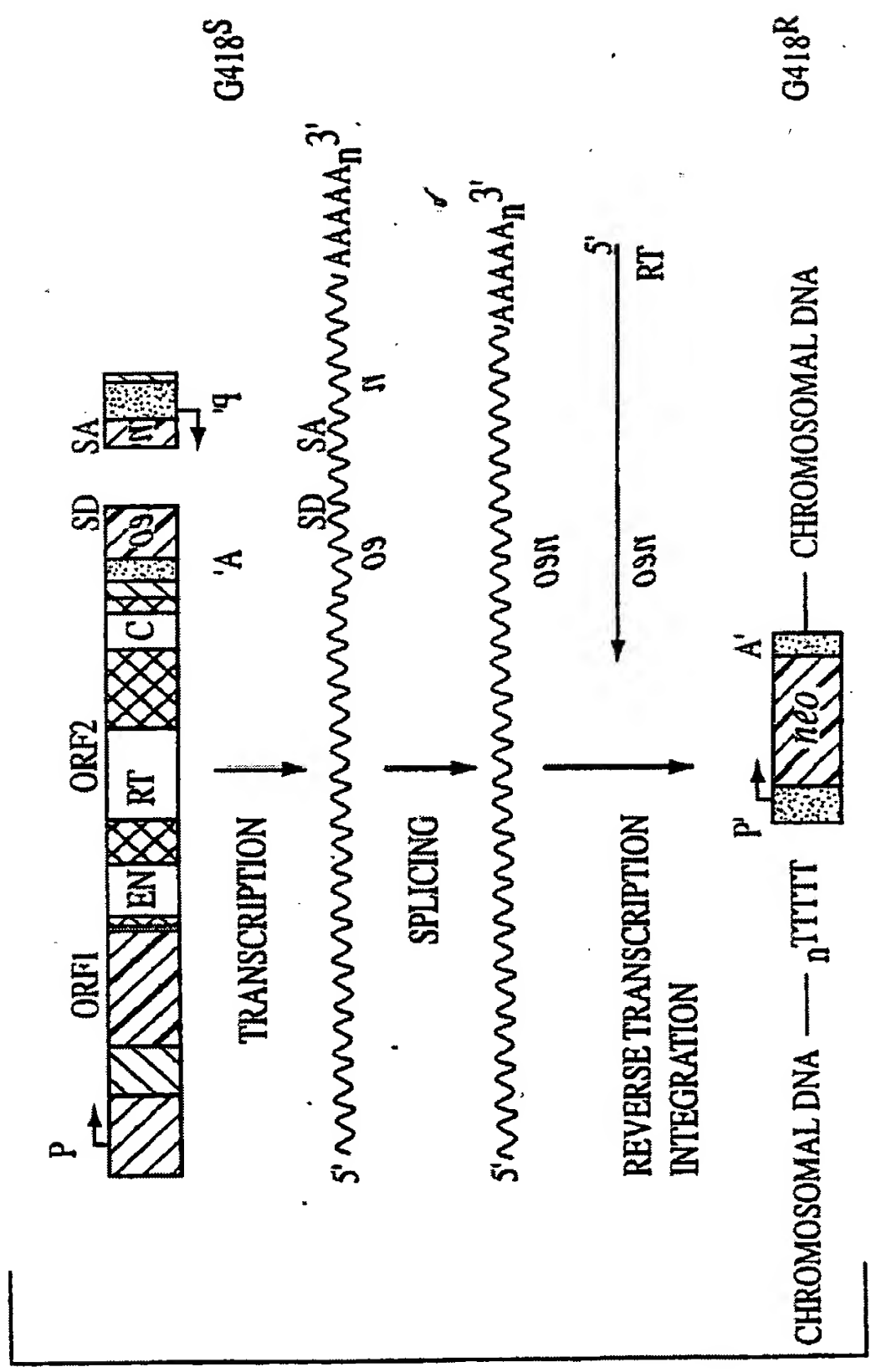

P
ORF1
EN
ORF2
RT
C
SD
09
'A
TRANSCRIPTION
5'
SD
09
SA
11
SPLICING
5'
09 11
REVERSE TRANSCRIPTION
INTEGRATION
09 11
5'
RT
CHROMOSOMAL DNA
n TTTTT
P'
neo
A'
CHROMOSOMAL DNA
G418S
G418R

FIG. 18A

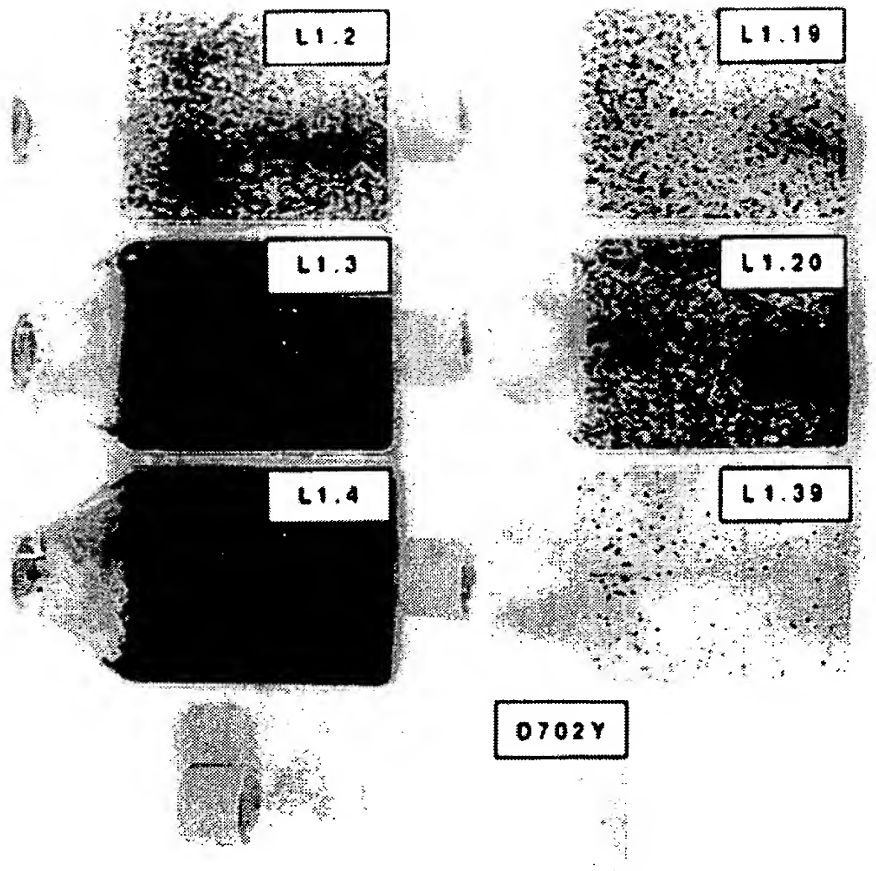



FIG. 18B